ARTIFICIAL CULTURE OF MARINE SEA WEEDS IN RECIRCULATION AQUARIUM SYSTEMS

JOHN A. STRAND, JOSEPH T. CUMMINS AND BURTON E. VAUGHAN

Biological and Medical Sciences Division, U. S. Naval Radiological Defense Laboratory, San Francisco, California 94135

Purely artificial sources of sea water at present are still totally inadequate to facilitate normal and prolonged growth of any of the more exacting marine sea weeds (Provasoli, 1963, p. 9). Enriched sea water techniques, however, have provided a suitable means to establish speciation and life-cycle determinations of several highly organized forms. In 1934 Föyn successfully cultured *Ulva lactuca* through a complete life-cycle in enriched media, using "Erdschreiber" sea water with N, P, and soil extract. Subsequent studies on artificial culture of *Ulva* and related genera have developed through empirical refinement of this basic technique.

Provasoli (1963, p. 11) was able to obtain nearly natural morphological development, *i.e.,* a flat blade-like thallus 2–3 cm. long, in sporelings of *Ulva lactuca* only with initial samples of enriched sea water. Subsequent samples enriched in the same manner yielded varying results. The inability to provide for sustained growth and development in a reproducible, synthetic medium has thus greatly restricted our understanding of salient ecological and physiological processes involved in growing marine sea weeds. Specific disadvantages of older enrichment methods are obvious, and they include: significant variability in micro-element composition of different sea water samples; reliance upon heat sterilization to provide contaminant-free media, often resulting in precipitation of essential additives; and variability in soil extract samples utilized.

It is evident that current methods for simulation of natural conditions are far from adequate. Information available provides exhaustive description of the uses of trace elements, growth regulators, and vitamin sources, but specifically fails to provide fresh insight into the complex of physical, chemical, and biological factors and the way in which they alter the bathing medium. In many respects oceans are relatively constant biological environments, for example, with respect to dissolved gases, salinity, pH, temperature, and illumination (Provasoli *et al.,* 1957). Wider variations are evident in littoral or estuarine waters, but again, transitions are probably gradual and seasonal under most natural conditions. In a laboratory situation these properties can be controlled readily. Biotic interaction and micro-element composition present additional complications, but in principle at least can be brought under laboratory control. For the work reported here, a closed-system approach was implemented to facilitate systematic study of essential parameters.

Materials and Methods

Marine sea weeds utilized in present investigations were gathered from Monterey Bay, Moss Beach, Point Reyes, and San Francisco Bay. Most species col-

lected were accurately identified in the field. Verification of closely related forms was achieved through standard histological technique. A fixing fluid consisting of 1 g. chromic acid, 1 ml. propionic acid in 90 cc. sea water proved to be satisfactory for most marine algae. Delafield's or Harris' hematoxylin was a suitable stain for most preparations; an appropriate counterstain of erythrosin B, orange G (0.8%) or fast-green (Johansen, 1951, pp. 359, 361) was also used.

Transplants, prior to placement into aquaria, were rinsed in fresh sea water and pesticide, using Lindane (hexachloro-cyclohexane), 5 ppm. for 15 minutes,

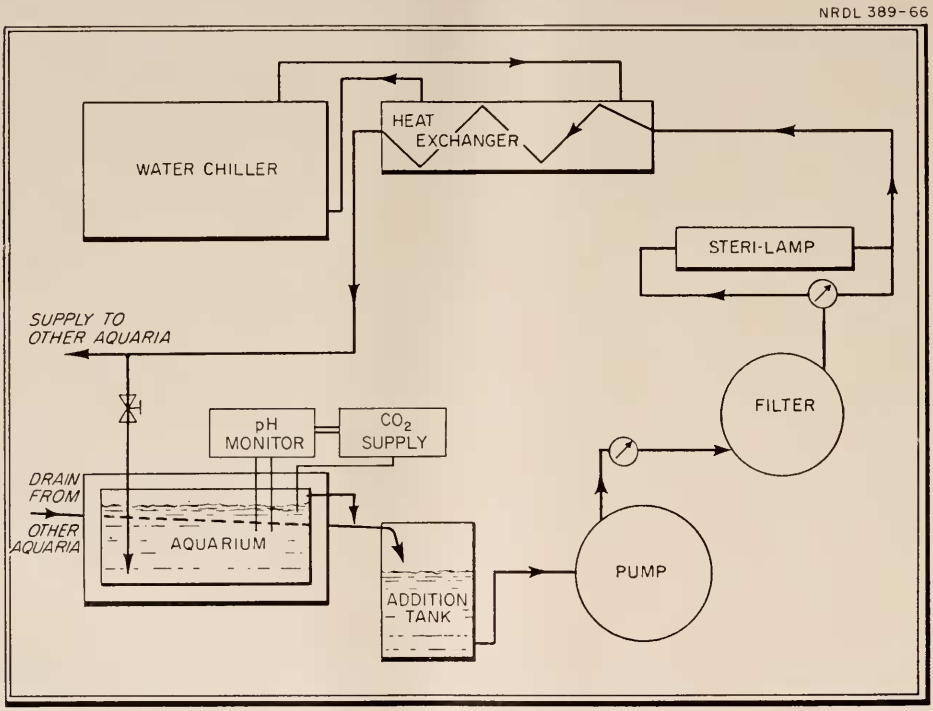

FIGURE 1. Flow diagram for recirculation aquarium system: temperature $16 \pm 1°$ C.; salinity $25 \pm 3‰$; total filtration from 4 to 10 μ; pH 7.9 ± 0.3; recirculation 190 L./min.; sea water replacement 4% per day. Details described in text.

to kill resident herbivorous gastropods and polychaetes. Polarographic measurements at the surface of *Ulva* after 24 hours exposure indicate that Lindane at concentrations of 650 ppm. did not appreciably inhibit oxygen evolution (report in preparation).

Of eight requirements to obtain optimal growth, five were adjusted empirically: (1) micronutrient enrichment, (2) CO_2 as a carbon source, (3) salinity and pH stabilization, (4) light source of suitable intensity and spectral quality, and (5) agitation and aeration. Additional requirements were necessitated due to the recirculation of sea water within the system: (6) nonmetallic construction, (7) filtration and sterilization, and (8) sea water replenishment. A schematic diagram of

the closed-recirculating aquarium system is shown in Figure 1; its components will be described separately below:

Fresh, sand-filtered sea water from off-shore was provided by Steinhart Aquarium, California Academy of Sciences, and this was enriched with phosphate, nitrate, and EDTA and other micronutrients. A mixture like that of Haxo and Sweeney (Provasoli *et al.*, 1957) provided a medium favorable to growth of *Ulva lobata* and related species, except that thiamin, biotin, and B_{12} were substituted for soil extract (Table I). Tris (hydroxymethyl) aminomethane buffer, pH range 7.5–8.5, also was added at 0.3 part per thousand. The salinity of fresh sea water with its micronutrients then was adjusted to 25 ± 3.0 parts per thousand. Daily replacement was maintained at the rate of 4% of the total volume of the aquarium system.

TABLE I

Sea water enrichment mixture

KNO_3	20.0 mg.
K_2HPO_4	3.5 mg.
$FeCl_3$	0.097 mg.
$MnCl_2$	0.0075 mg.
Glycerophosphate di-sodium pentahydrate	1.0 mg.
EDTA	1.0 mg.
B_{12}	1.0 μg.
Thiamin HCl	0.2 mg.
Biotin	1.0 μg.
Tris (hydroxymethyl) aminomethane	30.0 mg.
Fresh off-shore sea water	75.0 ml.
Distilled water	25.0 ml.

Vitamins, organic phosphate, and Tris buffer not included in Haxo and Sweeney formulation (1955).

The recirculating aquarium system was constructed of fiberglass, polyvinyl chloride (PVC), hard rubber, plastic, and glass, in order to reduce to a negligible degree contamination by undesirable metallic ions.

Water entering the closed system passed initially through a 20 μ mesh prefilter within the addition tank. Water leaving the addition tank passed through the pump (Duriron Company, Inc.) into a filter column containing a non-impregnated cellulose cartridge. The cartridge was designed to remove particles within a 4 to 10 μ range (Hilliard-Hilco Corp.). Efficient filtration was achieved at flow rates in excess of 190 liters per minute, and at pressures not exceeding 60 psi. Temperature control at 16 ± 1° C. was maintained by a two-stage system. The initial level was a heat exchanger in which 10–20% ethylene glycol in water was cooled with freon refrigerant by a 5-ton capacity compressor (Dunham-Bush, Inc.). The ethylene glycol solution in turn exchanged heat through an impervious graphite shell and tube heat exchanger, the latter of which circulated aquarium water (National Carbon Co.).

Alkalinity was monitored continually, and it was automatically stabilized at pH 7.9 ± 0.3 by continuous metering of CO_2 gas. For this purpose, standard pH electrodes were placed just below surface water. A pH meter (Beckman, Model

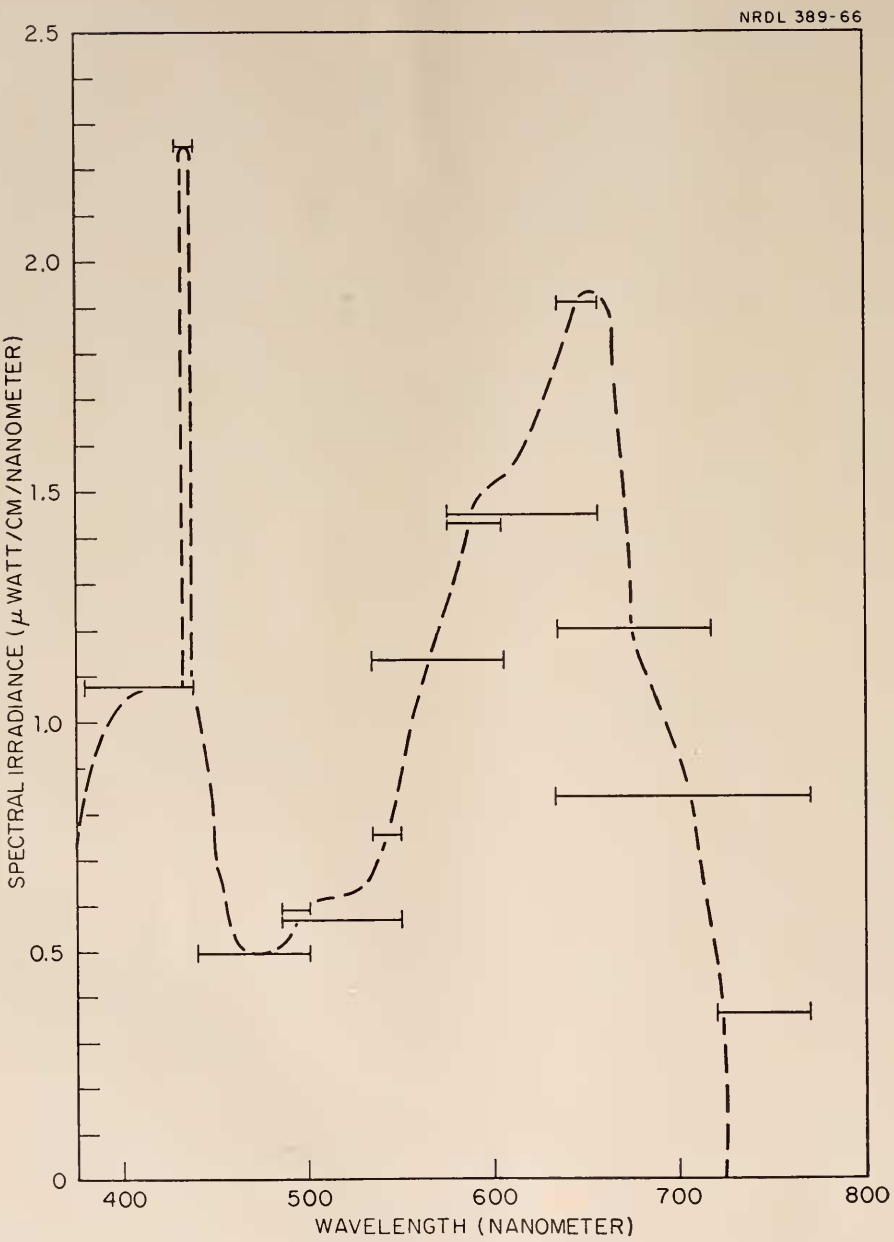

FIGURE 2. Spectral energy distribution (S.E.D.) at water surface (dotted line); energy detected through wide and narrow band-pass interference filters (horizontal bars). S.E.D. curve was interpolated, amounting to approximately 3.7 kilolux as the integrated area from 375 to 725 nanometers. For combined source, using 40 watts Gro-lux and 40 watts warm white (fluorescent). Details in text.

H-2) was modified by replacing the indicating meter with a meter relay switch. This switch activated a solenoid valve on the CO_2 cylinder when pH increased. Gaseous CO_2 entering the system in this manner not only enhanced pH stabilization but also provided an additional carbon source for photosynthesis.

Contaminating bacteria, protozoa, and diatoms were controlled through use of an ultraviolet sterilization source. This unit was constructed entirely of PVC and quartz glass tubing, discharging radiations in the 2537 Å range (Aqua-Fine Corp.). The unit efficiently killed biotic contaminants after 24 hours of recirculation, at flow rates in excess of 190 liters per minute. Elimination of biotic contaminants was confirmed bacteriologically.

Overhead lighting was provided for each aquarium. Two 40-watt, fluorescent lamps, 1 Gro-lux (Sylvania), and 1 Warm-white (General Electric), were placed in a standard white, painted reflector at the spacing recommended by the manufacturer (Mpelkas, 1964a). This, when located 50 cm. above aquarium top, yielded an irradiance almost entirely in the visible region, amounting to 3.7 kilolux at the water surface (Fig. 2). Energy emission of the Gro-lux lamps follows closely the absorption spectrum of chlorophyll pigments with peak energy output in the 440–460 and 660–680 nanometer range (Mpelkas, 1964b). Peak emission of the Warm-white lamp occurs within the mid-region of the action spectrum, 490–590 nanometers (Mpelkas, 1964b) fitting the absorption spectra of accessory pigments. Spectral energy distribution for the combined sources at 50 cm. is reported in Figure 2. This was determined using narrow band pass interference filters (Set 60; Optics Technology, Inc.) in line with an optical power meter (Model 610; Optics Technology, Inc.). Response characteristics of the meter-filter system were standardized against a known source for spectral irradiance. The photoperiod was maintained automatically, and was varied according to conditions to be described at a later point. Culture tanks were installed in a windowless room, devoid of natural sunlight.

Results

Taxonomy and natural history of Ulva lobata

Thalli, like those described by Setchell and Gardner (1920) and Smith (1944) were found along the California Coast; they attained moderate size, nearly 50 cm. tall, 20–30 cm. broad, and were usually rich green in color. The plants observed were saxicolous and occasionally epiphytic, and they were found in the mid-littoral zone between 2.0- and − 2.0-ft. tide levels. Blades were membranaceous, broadly expanded, deeply divided, and slightly ruffled at margins. The thalli gradually narrowed to a stipe-like holdfast. Holdfast structures were perennial; blade portions, annual. This species differed from the closely related *Ulva expansa*, primarily in extent of division and size of blade. The latter was not deeply divided and was observed to attain a length in excess of 150 cm. Like those of all species of the genera, the blade of *Ulva lobata* was distromatic. It varied from 40 μ thickness at the margin to 90 μ in the more central portion. Cells as examined microscopically are shown in Figure 3.

The literature indicates that: reproduction follows an alternation of identical asexual and sexual generations; each fertile cell of the diploid sporophyte is capable

of producing 8 or 16 quadri-flagellate zoospores; meiosis occurs during the first and second divisions, the zoospores developing into haploid gametophytes; and that the gametophyte generation is heterothallic and anisogamous (Smith, 1944). Gametes released by mature plants were measured in the present study by stage

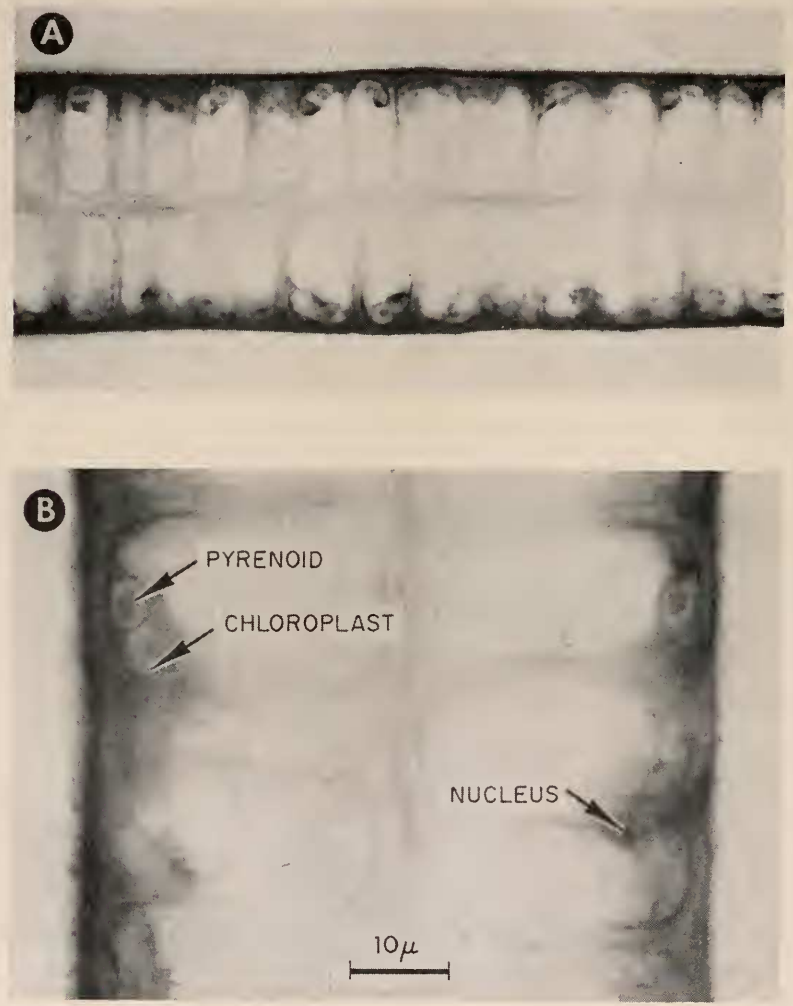

FIGURE 3. *Ulva lobata*, cross-section, H&E stain. Cells appear tightly packed with walls of adjacent cells confluent to form a rigid matrix. Note distromatic organization. (A) × 760, (B) × 1900.

micrometer, verifying reported descriptions of anisogamy. Male gametes here measured 2.0–3.5 × 5.5–7.0 μ in size; female gametes measured 2.5–4.0 × 6.0–8.0 μ. Each was ovate or pear-shaped, showing a conspicuous eyespot and chloroplast, and each was bi-flagellate.

Gametes and zoospores were observed to be discharged through a small pore on the external border of marginal cells. The exit pore was not observed until the onset of sporulation. Instantaneous with release, gametes or zoospores collected into clumps, containing often as many as 50–100 cells. This clumping was somewhat larger than that reported by others (Smith, 1947). Gametic union occurred during clumping, pairs fusing side-by-side or anteriorly end-on-end. Clumps disintegrated within 2 to 3 minutes, and the quadri-flagellate zygotes remained motile for several hours. Zoospores were observed to remain motile longer, *e.g.,* 4 to 5 hours.

Available literature (Smith, 1947) shows that fruiting or sporulation of both gametophyte and sporophyte generations occurs at predictable 28-day intervals, but only during the spring tides of the lunar month, as observed during summer months. Gametophytic plants sporulate early during the series of spring tides, while sporophytic plants liberate zoospores late during the series. Thalli of both generations are usually found closely associated and in approximately equal numbers. Gametes have been reported to germinate parthenogenetically (Smith, 1947; Moewus, 1938; and Yamada and Saito, 1938).

Aquarium development and differentiation of Ulva lobata

Fertile thalli of *Ulva lobata* were placed into plastic bags containing fresh sea water and transported to the laboratory in ice chests maintained at 10–15° C. Mature plants transported in this manner often discharged swarmers (gametes or zoospores) within a few hours, as they also did when placed into fresh sea water of normal seasonal temperature. The slightest change in environmental conditions often stimulated sporulation, for example decreasing temperature, desiccation or stimulation by intense light. Contents of bags showing spore liberation were mixed and gently agitated for one hour to insure fertilization. The resulting zygospores were poured into a 225-liter culture tank and water turnover was reduced to allow their implantation on the aquarium bottom.

Infertile thalli usually developed to maturity within a few days after placement into laboratory aquaria. Occasionally blades would sporulate spontaneously, usually 2–3 weeks after transplant. Even as late as December or January infertile thalli were seen to develop mature reproductive cells and could be stimulated to release swarmers.

Development of sporelings was followed on a daily basis and compared to existing accounts of similar species. Photomicrographs were prepared to record the significant stages of differentiation not previously described for *Ulva lobata* (Fig. 4). Development of the sporophyte generation will be described below.

One to two days after fertilization and implantation, zygotes became more spherical, approximately 6–8 μ in size. Two eye-spots and a single pyrenoid were still recognizable (Fig. 4A, B). In from 2 to 4 days, zygotes increased their size to 8–10 μ and showed a thin, clear membrane which gradually thickened as the onset of germination approached. Germination occurred in from 6 to 7 days and the eye-spots at this stage were no longer visible. At 9 to 10 days, the sporeling appeared as a slender filament of 8–10 cells, 40–45 μ in length, since division only occurred along the transverse axis (Fig. 4C). At this time, the basal cell sometimes elongated to form a primary rhizoid. At 14 days, and at nearly 100 μ in

length, the first cellular divisions along the longitudinal plane of the filament occurred (Fig. 4D); secondary rhizoids were then observed to develop. Through continued longitudinal divisions, at 40 days, the sporeling represented the flattened membranaceous structure of the mature thallus. At 60 days, the young plant was 4.5–5.0 cm. in length and 1.5–2.0 cm in breadth (Fig. 4E). Although the young sporophytes were of sufficient size and maturity to liberate zoospores, subsequent development of the gametophyte generation was not followed. In these

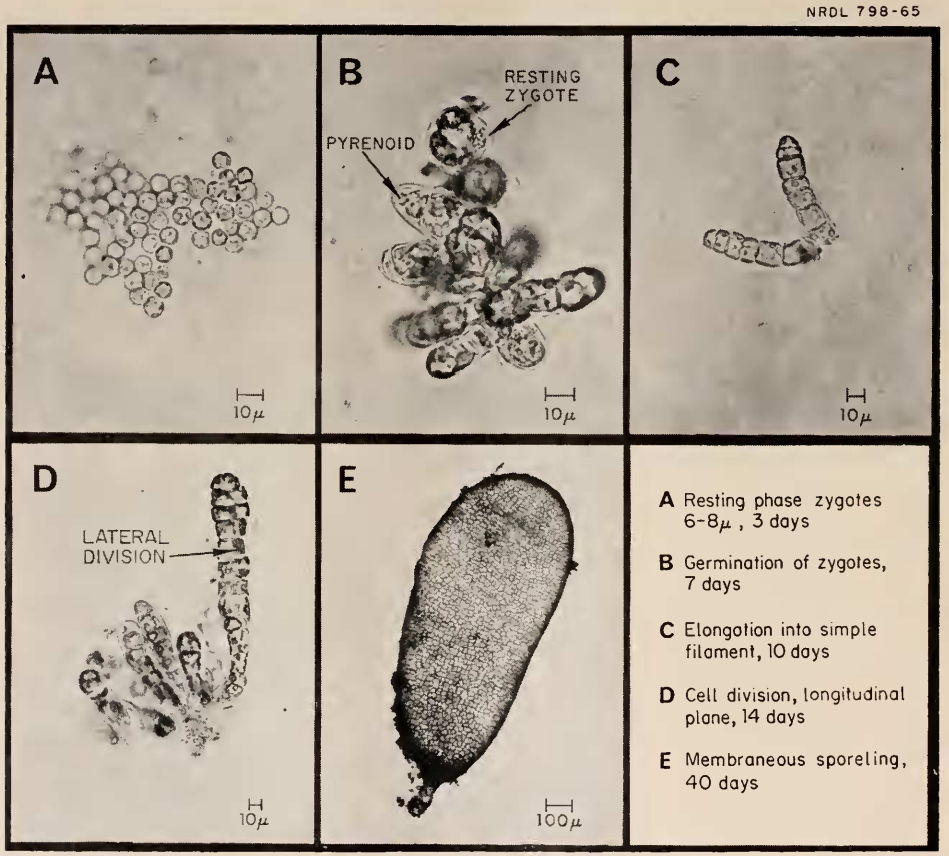

FIGURE 4. Photomicrographs showing development and differentiation of *Ulva lobata* sporelings in recirculation aquarium system.

studies it was not possible to distinguish morphological differences in the development of *Ulva lobata,* when compared to the closely related species, *Ulva (lactuca)* (Miyake and Kunieda, 1931) or *Ulva pertusa* (Yamada and Saito, 1938). Normal elongation and subsequent differentiation were clearly demonstrated in Figure 4. Deformities such as the acute twisting and bulging of *Ulva lactuca* filaments, described by Rea (1964), were not evident.

Longitudinal division of filaments of *Ulva lobata* occurred in approximately 10–14 days here, whereas in a study of *Ulva pertusa,* it occurred no earlier than

30 days after germination (Yamada and Saito, 1938). Also, in the present study sporelings were nearly ten-fold larger at 45 days, compared to those of *Ulva pertusa* as measured at 60 days. These differences probably do not indicate different species characteristics, but rather attest to the adequacy of the present culture conditions. It seems likely that earlier accounts of sporeling development were hampered by less adequate growth conditions.

Laboratory-maintained Ulva transplants

The genus *Ulva* demonstrates a marked seasonal periodicity with respect to both vegetative and reproductive growth, and this is mediated undoubtedly through both environmental and less understood internal factors. Among the former, duration of photoperiod and water temperature are of particular interest.

In general, vegetative growth in *Ulva* is observed to begin slowly in early Spring with the known lengthening of photoperiod and rise in water temperature. Maximum attainment of both vegetative and reproductive processes is reached by mid-summer. The growth response gradually diminishes by late summer, the thallus dying back to where only the holdfast and a small portion of the leafy blade survive the winter months. This attrition seems to be determined by changes in duration of photoperiod and environmental temperature. It affects both gametophyte and sporophyte generations alike.

Care was exercised during collection of specimens not to damage or disrupt holdfast structures. Usually small plants, less than 20 cm. tall and firmly anchored to small rocks or pebbles, were selected. Thalli in which holdfasts were carefully cleaved from attachment occasionally would continue differentiation of this structure and subsequently reattach on aquarium bottoms. Usually samples of *Ulva lobata, Ulva lactuca,* and *Ulva linza,* with holdfasts intact, transplanted better; however, this was not true of *Ulva expansa.* In the latter species, free-living thalli frequently break away from the holdfast and continue to grow, if free-floating in more quiet backwaters (Smith, 1944).

Seasonal rhythm was preserved during laboratory culture and was paramount in determining transplant longevity (Table II). If viable plants were removed for transplant early during the growth season, that is, during May or June, they were usually adequately maintained for three months or more, comparable to the natural seasonal growth. Thalli introduced to laboratory culture late during the growth season exhibited a less extensive vegetative development or none at all. Exposure to those environmental conditions correlated with maximal vegetative and reproductive development did not alter the onset of senescence; *e.g.,* water temperature (surface) 16–18° C. and photoperiod 13–15 hours, which are mean summer conditions in Monterey Bay. Long-day illumination, 13–15 hours, under present methods of culture favored longevity of *Ulva* transplants removed from the sea during the months of May, June and July (Table II). Thalli removed during late summer, August and September, were maintained only if the photoperiod was decreased to 10–12 hours. Exposure to photoperiods above 16 hours or below 10 hours caused a gradual degeneration of tissues in 7–10 days. Exposure to continuous light produces a rapid shriveling and hardening of the thallus, evident in 48 hours. Microscopic examination of tissues illuminated continually for 96 hours revealed a marked dissolution of mucilage material associated with the free

TABLE II

*Effect of photoperiod and other lighting conditions on longevity; summarized data for Ulva transplants maintained in recirculation aquaria**

Species	Transplants or sporel. (N)	Natural photoper. (hr.)	Artificial photoper. (hr.)	Source of illum.	Irrad. level (kilolux)	Longevity‡
May–Ju. collections:						
U. lobata	12	14.0	13–15	GL+WW	3.7	3 mo.
U. lobata	12	14.5	16–18	GL+WW	3.7	1 wk.
U. expansa	12	14.5	16–18	GL+WW	3.7	1 wk.
U. lobata	4	14.5	continuous	GL+WW	3.7	48 hr.
U. expansa	4	14.5	continuous	GL+WW	3.7	48 hr.
U. lobata	12	14.5	13–15	GL+WW	7.4	1–2 wk.
Oct. collections:						
U. lobata	12	11.5	10–12	GL+WW	3.7	2 mo.
U. expansa	12	11.5	10–12	GL+WW	3.7	2 mo.
Sporelings, U. lob.	300Δ	11.5	13–15	GL+WW	3.7	3 mo.
Sporelings, U. lob.	300Δ	11.5	13–15	GL+WW	7.4	3 mo.
U. lobata	12	11.5	13–15	GL+WW	3.7	1–2 wk.
U. expansa	12	11.5	13–15	GL+WW	3.7	
U. lobata	12	11.5	8	GL+WW	3.7	1 wk.
U. expansa	12	11.5	8	GL+WW	3.7	1 wk.
U. lobata	24	11.5	10–12	DL+DL	3.7	1–2 wk.
U. expansa	24	11.5	10–12	DL+DL	3.7	1–2 wk.
U. lobata	24	11.5	10–12	CW+CW	3.7	1–2 wk.
U. expansa	24	11.5	10–12	CW+CW	3.7	1–2 wk.
Dec.–Jan. collections:						
U. lobata	24	10.0	10–12	GL+WW	3.7	2 mo.
U. expansa	24	10.0	10–12	GL+WW	3.7	2 mo.

* Nutrient factors, salinity, pH and other conditions are constant; see text.

† Forty-watt fluorescent tubes: GL = Gro-Lux; WW = Warm-white; DL = Daylight; CW = Cool white.

‡ Bleaching and autolysis first evidenced; for continuous illum., dissolution of mucilaginous envelope and shriveling of thallus were evidenced at 48 hours.

Δ Number estimated from area and number in microscopic field.

cell border surfaces of the blade. Comparable tissue breakdown occurred if light intensities exceeded 3.7 kilolux, although sporelings of *Ulva lobata* tolerated intensities at a level of 7.4 kilolux, as well as a longer photoperiod.

Unbuffered aquarium waters showed the well known rapid decrease in hydrogen ion concentration when transplants were illuminated (Shelbourne, 1964, p. 29). During dark periods pH values fell, but not to initial levels: thus, total alkalinity gradually increased within 5–7 days to pH values in excess of 9.0. Sustained

alkalinity of this magnitude usually resulted in a progressive degeneration of transplanted thalli. Comparable effects resulted if pH values were maintained below 7.5.

Discussion

Possible seasonal change in the water occurring along the mid-California coast did not affect the success of present cultures. Sterilization of media by autoclaving was not necessary to avoid gross biotic infection, as effective control was achieved by ultraviolet irradiation. This also permitted use of a wider salinity range, as well as greater concentrations of both inorganic and organic constituents precipitable by heat sterilization. Vitamins, chelators, and other organics could be added to media precisely with results comparable to those using soil extracts. While the B vitamins, thiamin, B_{12}, and biotin, have been shown to stimulate growth of many unicellular algae including diatoms their role is less understood for marine sea weeds. Of the Rhodophyceae, *Goniotrichum, Nemalion, Antithamnion*, and *Bangia*, all are found to utilize one or more of the B vitamins in laboratory culture (Provasoli, 1963, p. 13). Kylin (1942) reported enhanced growth of both *Ulva* and *Enteromorpha* with thiamin at an optimal concentration of 10 mg./liter. None of the Phaeophyceae thus far studied are known to require these additives, but since insufficient information is available, it seemed inadvisable to omit these constituents.

Tris (hydroxymethyl) aminomethane at 0.03% concentration in the system buffers adequately between pH 7.5 and 8.5. Thus, pH levels in the aquaria were stabilized at 7.9 ± 0.3, with gaseous CO_2 (1–10%) bubbled through the medium intermittently. Provasoli (1957) indicated that Tris (0.1%) was not inhibitory to the most sensitive of marine algae, and in the present work, comparable concentrations were not inhibitory to either transplants or developing sporelings of *Ulva*. EDTA at a final concentration of 3×10^{-5} *M* is sufficient to bind the trace elements in sea water (Johnston, 1964). EDTA has the added advantage of being metabolically inactive for most organisms, and it evidently does not promote growth of contaminating biota within non-sterile media (Hutner *et al.*, 1950).

In maintaining proper alkalinity levels, ultraviolet sterilization and microfiltration supplanted chemical buffering by eliminating bacterial and other organic growth which would otherwise acidify the medium. Many sea weeds are found to be tolerant of wide fluctuations in pH, due primarily to tidal influence. Blinks (1963) demonstrated that photosynthetic rate decreased by 50% only, when fronds of *Ulva* and *Enteromorpha* were maintained for about 6 hours at pH 9.8 or above. Kylin (1927) indicated that certain intertidal sea weeds survived for 1–3 days within the pH range of 6.8 to 9.6. From the present studies, it would appear that *Ulva* and related genera are more closely restricted in pH requirements than might first be suspected, and that even high ranges as encountered in isolated tide pools would be inhibitory if sustained longer than normal tidal influence allows.

Typically *Ulva lobata* and related species are long-day, short-night plants. At an irradiance of 3.7 kilolux, illumination for 13–15 hours and uninterrupted darkness for 9–11 hours favor longevity of transplants. In nature, both vegetative and reproductive development reach maturity during similar long-day seasons. Also it is noted that lunar periodicity governs the release of gametes and zoospores, undoubtedly correlated to the 24-hour light-dark cycle (Smith, 1944).

From our field observations, water temperature seemed to have a greater effect on the rapid maturation of gametes and zoospores than duration of photoperiod. Although approaching maximum vegetative growth in late spring, fertile thalli were not evident until water temperatures reached summer levels, 16–18° C. It followed that incidence of fertile plants decreased abruptly during early September as water temperatures within the vicinity of collection sites fell below 16° C. That the response was a consequence of temperature change and not a consequence of the shortened photoperiod was shown by the following observation. Placing infertile plants collected as late as December or January into aquaria maintained at 16–18° C., with an artificial photoperiod of 9–10 hours, did induce subsequent maturation and sporulation (data not reported).

The influence of temperature on reproductive growth of many plants is closely interrelated with photoperiodism. Depending upon the species of plant and other conditions, temperature may enhance or oppose the effect of photoperiod on reproductive maturity (Meyer and Anderson, 1952, p. 681). Temperature changes may initiate synthesis or breakdown of hormonal compounds, rates of translocation, and relative effectiveness of specific morphogenic change (Meyer and Anderson, 1952, p. 681). Aquatic plants such as *Ulva* perhaps have biochemical similarity to terrestrial plants, since comparable phyto-hormone responses are present (Provasoli, 1957, 1958), and since metabolic effects can be triggered by photoperiod and temperature changes like those known to depend on phyto-hormone systems.

Scant information is available concerning either the intensity levels or the spectral quality essential to artificial maintenance of marine sea weeds. An intensity range of 1.1 to 5.4 kilolux has been reported for Rhodophyceae (Provasoli, 1963, p. 10; Iwasaki, 1961). Föyn (1934, 1960), however, with *Ulva* did not report intensity ranges. Other investigators have simply exposed cultures to natural sunlight. Investigations by Dellow and Cassie (1955) on the littoral zonation in caves demonstrated the adaptability of such forms as *Cladofora* to grow normally at low intensity levels, in the range of 0.005 to 0.250 kilolux. In similar experiments, the intertidal alga, *Ulva lobata,* could survive 5 hours exposure to direct sunlight (104 kilolux) (Biebl, 1952). However, in the natural environment, constantly changing intensities, attenuation of red, and scattering of blue wave-lengths, are all complicating factors.

While the energy distribution of the Sylvania Gro-lux fluorescent lamp approximated a photosynthetic action spectrum (Mpelkas, 1964b), the combined sources here used provided a better spectral balance for growth and differentiation. Haxo and Blinks (1950), in comparing light absorption and photosynthetic action spectra in *Ulva* and *Monostroma,* demonstrated that higher rates occurred in spectral bands corresponding to absorption by chlorophyll A, at 435 and 675 nanometers. In our studies, when *Ulva* transplants were illuminated at comparable intensities but with Gro-lux lamps alone or with Cool-white or Daylight lamps, the transplants gradually bleached and decayed. These adverse changes presumably were due to inadequate energy emission in the red spectral regions when the sources indicated were used individually (Mpelkas, 1964; Haxo and Blinks, 1950). For the combined light sources finally adopted, abrupt drop in energy distribution below 400 and above 725 nanometers (Mpelkas, 1964b) lessens concern about possibly unfavorable emissions, *e.g.,* mercury lines, ultraviolet and infrared wave-lengths.

With little modification, the basic system has facilitated cultures of other marine forms, *e.g.*, sea weeds such as *Porphyra perforata, Polyneura latissima* and *Schizymenia pacifica,* and diatoms such as *Ditylum brightwelli, Nitzchia angularis* and *Navicula* (sps. und.). It has also permitted successful culture of such animal forms as protozoa, annelids, molluscs and crustaceans.

The authors are grateful for the kind assistance of Mr. Clay P. Butler of this Laboratory, who standardized their tungsten source against NBS calibrated lamp QL-50 (tungsten).

Summary

Ulva as either sporeling or transplant could be cultured for periods of three months in closed recirculating aquarium systems. Early development of *Ulva lobata* sporelings proceeded normally and rapidly under conditions imposed and compared to closely related species, *i.e., Ulva pertusa* and *Ulva lactuca.* A modified Haxo-Sweeney enrichment was used, substituting B vitamins and organic phosphate for soil extract. Continuous flow ultraviolet sterilization and microfiltration were provided. The pH was maintained automatically at 7.9 ± 0.3, using Tris buffer and gaseous CO_2. Improved fluorescent illumination for 13–15 hours favored culture of sporelings and summer transplants. Irradiance was confined to the spectrum lying between approximately 380–725 mμ, and amounted to 3.7 kilolux. From field observations, photoperiod appeared closely correlated to initiation of vegetative growth during early spring. Water temperature seemed to have a greater effect on the rapid maturation of gametes and zoospores.

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